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INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: Mr. J. L. Charles Date: October 29, 1981

From: R. W. McCuen

Subject: Project Charge Number 6904 (Biochemical Methods Development and Utilization)--Plans and Objectives for 1982

The objectives of this project are similar to those of Project Charge Number 6906 before it was split. These are:

a) to develop short-term mammalian cell *in vitro* assays to evaluate the potential biological effects of cigarette smoke products or potential additives.

b) to conduct research investigations designed to understand the nature of cigarette smoke product activity in each *in vitro* assay.

The mammalian cell bioassays we will develop and/or use to accomplish these objectives are described below.

1. L5178Y Mouse Lymphoma Assay

Investigators: D. Ayers (Associate Scientist B) and M. Penn (Scientist) functioning as a team.

The two major objectives, plans to attain these objectives, and projected time frames in which the work will be done are as follows:

A. Observe a negative response(s) in the $TK^{+/-}$ mutation assay in our laboratories. We will test pure chemical compounds KNOWN to produce no bioactivity (other than toxicity) *in vitro* and/or *in vivo*. Such compounds as acetone, pyrene, ethylene glycol, sucrose, and gibberellic acid will be tested. [A revision of our SOP definition of a positive and/or negative response in this assay may be necessary at the conclusion of this work.]

First Quarter and Continuing

B. Elucidate some of the filler/smoke parameters associated with the activities of various tobacco or nontobacco smoke products in the $TK^{+/-}$ mutation assay. This will involve personnel of Project Charge Numbers 6908 and 6910. We will characterize (with and without microsomal activation, dose-response curves, *etc.*) MS (IT CSC, VP and SIT) and SS bioactivity from the nitrogen-free high organic, low ash NTSM--LTF-5E cigarette.

First and Second Quarters

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The above results will be documented in a special report. An attempt will then be made to change the basal activity level of the various LTF-5E products stated above by altering the filler composition. Such alterations *might* include the addition of nicotine, urea, or specific amino acids.

Third Quarter and Continuing

[NOTE: As part of this objective and as a carry over from some work done in 1981, we plan to test the VP of selected cigarettes for their L5178Y bioactivity in relation to their contents of certain carbonyl type compounds (acetaldehyde, formaldehyde, acrolein, diacetyl, and methyl vinyl ketone). Thus, we will be in a position to determine if a quantitative relationship exists.]

Second Quarter

C. From conversations with J. L. Charles, we expect to receive some requests for testing CSCs and/or potential cigarette additives in 1982. This work will be conducted on a high priority basis.

Continuing

2. V79 Chinese Hamster Lung Cell Assay

The two major objectives, plans to attain these objectives, projected time frames in which the work will be done and the principal investigator are as follows.

A. ESTABLISH the mutational endpoint (HGPRT marker) of this cell system in our laboratories. The work will be conducted by L. Weissbecker (Research Scientist). [Eventually, it is our goal to replace the L5178Y/TK+/- mutation assay with the more versatile, multi-endpoint V79 bioassay.] Initially, we plan to learn general cell handling techniques, cell characteristics, *etc.* Ethyl methanesulfonate (EMS) will be tested as a nonactivation positive control using 6-thioguanine (6-TG) as a selective agent in order to establish dose-response curves, assay reproducibility, and variability.

First Quarter

Next, we plan to test a positive activation control, 7-12-dimethylbenz(a)anthracene (DMBA), using the same selective agent with microsomal activation in order to establish dose-response curves, assay reproducibility, and variability.

Second and Third Quarters

The above three quarters of work will be documented in a special report. Additional pure agents representing chemical classes other than polycyclic aromatics (DMBA) will be tested with microsomal activation as described above in an attempt to extend the second and third quarter results. The *completion* of this work will ESTABLISH this assay system in our laboratories and be in a position to use it for potential cigarette additive testing in 1983.

Fourth Quarter and Continuing

B. ESTABLISH the "promoter assay" endpoint of this cell system in our laboratories. The work will be conducted by R. McCuen (Associate Senior Scientist). [This is part of the multi-endpoint capabilities of this cell system as alluded to above.] This assay is based on the following observations. Wild-type Chinese hamster V79 cells (6-TG^S) reduce the recovery of 6-TG^Y cells when co-cultured at high cell densities in the presence of 6-TG. This reduction has been shown to occur because of intercellular communication (metabolic cooperation). Metabolic cooperation is inhibited by most known promoters, thus rescuing the 6-TG^Y cells. This assay is not based on the interaction of an active agent with DNA. Some evidence has been presented in the literature which shows that this assay can discriminate between tumor promoters possessing various degrees of *in vivo* potency. Initially, we plan to learn general cell handling techniques, cell characteristics, *etc.* Several spontaneously- and/or EMS-induced HGPRT^- (6-TG^Y) mutants will be obtained, pooled, and a cell stock prepared.

First and Second Quarters

The promoter assay will be conducted using HGPRT competent and HGPRT deficient V79 cells and the known promoter 12-O-tetradecanoyl phorbol-13-acetate (TPA) in an effort to establish dose-response curves, assay reproducibility and variability.

Third Quarter

Finally, we hope to test other positive (butylated hydroxytoluene and phenobarbital) and negative (phorbol) pure chemicals in this system to determine dose-response curves, assay reproducibility and variability. If successful, the V79 HGPRT promoter assay would be ESTABLISHED. These results would be documented in a special report.

Fourth Quarter and Continuing

SUMMARY OF PLANS AND OBJECTIVES FOR PROJECT CHARGE NUMBER 6904 FOR 1982

	Assay/Activity	Time
1.	L5178Y/TK$^{+/-}$ Mutation Assay	
	A. Negative control compound	first quarter and continuing
	B. Determinants of cigarette activity	
	LTF-5E	first quarter and continuing
	VP/carbonyl studies	second quarter and continuing
	Additive and CSC testing	upon request
2.	V79 Chinese Hamster Lung Cell Assay	
	A. Establish HGPRT mutational endpoint	
	Test EMS without S9	first quarter
	Test DMBA with S9	second and third quarters
	Document results	fourth quarter
	Test other classes of agents with S9	
	B. Establish HGPRT promoter assay endpoint	
	Learn cell handling techniques Obtain stock of HGPRT$^-$ cells	first and second quarters
	Conduct assay with TPA	third quarter
	Test other positive and negative controls Document results	fourth quarter

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